

REMARKS

The amendments to the specification insert SEQ ID NO:s and the sequence listing into the specification of the patent application. These amendments do not add new matter into the application. Accordingly, Applicants respectfully request entry of this amendment in its entirety.

Respectfully submitted,

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By 

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**MARKED UP VERSION ATTACHED TO AMENDMENT IN
SERIAL NO. 09/943,382**

Marked up version of paragraph [0231] starting at page 84, is below:

[0231] The kinase activity of various protein tyrosine kinases can be measured by providing ATP and a suitable peptide or protein tyrosine-containing substrate, and assaying the transfer of phosphate moiety to the tyrosine residue. Recombinant proteins corresponding to the cytoplasmic domains of the flt-1 (VEGFR1), KDR (VEGFR2), and bFGF receptors were expressed in Sf9 insect cells using a Baculovirus expression system (InVitrogen) and purified via Glu antibody interaction (for Glu-epitope tagged constructs) or by Metal Ion Chromatography (for His₆ (SEQ ID NO: 1) tagged constructs). For each assay, test compounds were serially diluted in DMSO then mixed with an appropriate kinase reaction buffer plus ATP. Kinase protein and an appropriate biotinylated peptide substrate were added to give a final volume of 100 μ L, reactions were incubated for 1-2 hours at room temperature and stopped by the addition of 50 μ L of 45mM EDTA, 50mM Hepes pH 7.5. Stopped reaction mix (75 μ L) was transferred to a streptavidin coated microtiter plate (Boehringer Mannheim) and incubated for 1 hour. Phosphorylated peptide product was measured with the DELFIA time-resolved fluorescence system (Wallac), using a Eu-labeled anti-phosphotyrosine antibody PT66 with the modification that the DELFIA assay buffer was supplemented with 1 mM MgCl₂ for the antibody dilution. Time resolved fluorescence was read on a Wallac 1232 DELFIA fluorometer. The concentration of each compound for 50% inhibition (IC₅₀) was calculated by non-linear regression using XL Fit data analysis software.

Marked up version of paragraph [0232] starting at page 85, is below:

[0232] Flt-1, KDR, and bFGFR kinases were assayed in 50 mM Hepes pH 7.0, 2 mM MgCl₂, 10 mM MnCl₂, 1 mM NaF, 1 mM DTT, 1 mg/ml BSA, 2 μ M ATP, and 0.42 μ M biotin-GGGGQDGKDYIVLPI-NH₂. (SEQ ID NO: 2) Flt-1, KDR, and bFGFR kinases were added at 0.1 μ g/mL, 0.05 μ g/mL, or 0.1 μ g/mL respectively.